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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
IVAYLO GENTSCHEV, ET AL.	: EXAMINER: FENSTERLE ET
SERIAL NO: 10/559,663	:
FILED: JUNE 21, 2006	: GROUP ART UNIT: 1645
FOR: CELLS USED AS CARRIERS FOR BACTERIA	:

DECLARATION UNDER 37 C.F.R. §1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

I, Joachim Fensterle state:

1. That I am a graduate of Biotechnology and received my diploma degree in the year 96.

2. That I have been employed by Hellenic Pasteur Institute for 3 years as a researcher in the field of vaccine development

3. That I understand the English language or, at least, that the contents of the Declaration were made clear to me prior to executing the same.

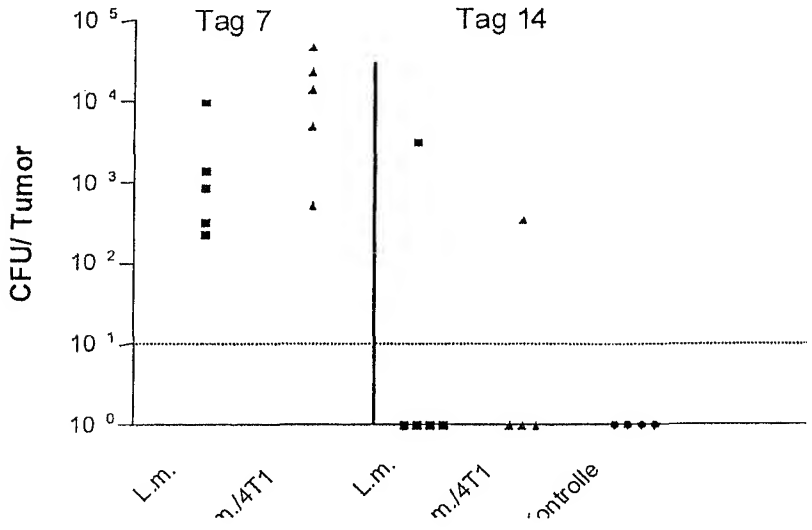
4. I am a named inventor of U.S. application serial no. 10/559,663.

5. The following experiments were performed by me or under my direct supervision.

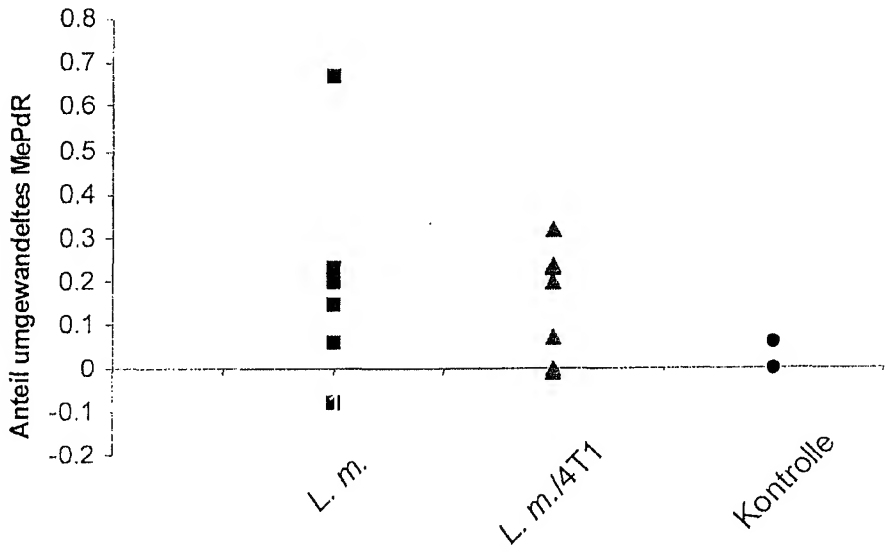
6. The following experiments demonstrate that transgenic listeria is enriched in tumor and expresses a functional prodrug-converting enzyme.

7. We used *Listeria* infected 4T1 mice that carry tumors (*in vivo* model).

8. The question was to demonstrate that transgenic bacteria expressing a functional prodrug converting enzyme delivered via cells are enriched in the tumor tissue and can functionally convert the corresponding prodrug in tumor tissue samples.
   The product of the conversion, 6-Methylpurine (MeP), is toxic to tumor cells and the sum of enrichment in the tumor tissue and successful conversion is therefore a direct correlate to the efficacy.
9. To answer these question, the following method was applied:
   a. A recombinant, attenuated Listeria strain (L. monocytogenes delta aroA) was constructed using standard molecular biology techniques encompassing a plasmid encoding the E.coli purinnucleotidephosphorylase (PNP) under the control of the CMV promoter active in eukaryotic cells (DNA delivery). This enzyme mediates the conversion of the prodrug 6-Methylpurine deoxyribose(MePdR) to MeP. The latter product is toxic for tumor cells.
   b. Animals were transplanted with 10^4 4T1 breast cancer cells. Tumors were allowed to grow up to a tumor diameter of approx. 0.5 cm before infection.
   c. Animals were infected IV with $1.3x10^6$ recombinant Listeria or $2.0x10^7$ bacteria in irradiated 4T1 cells.
   d. The CFU in the tumor tissue was determined by plating serial dilutions 7 days after infection.
   e. 7 days after infection, the tumor was excised and homogenized. Tumor lysates were incubated for 48 h with the substrate MePdR. After incubation, the substrate conversion into MeP was assessed by HPLC. The results are expressed as relative amount of formed MeP.
10. The results depicted in the following figure demonstrate that the recombinant Listeria strain is effectively delivered into tumor cells in this experimental system.



The following picture encompasses the enzyme activity 7 days after infection. This picture demonstrates that the bacteria delivered by the irradiated cells can deliver the DNA into the tumor tissue which, in turn is functionally transcribed in the eukaryotic target cells. As bacteria are both delivered into the tumor system by cellular carriers and the enzyme is active, the system is efficient for tumor therapy.



11. The undersigned declares further that all statements made herein are of his own knowledge are true and that all statements made on information are believed to be true. Further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Signature

November, 26th 2007
Date